

Clinical, hemato-biochemical and ruminal fluid changes pre- and post-treatment of induced lactic acidosis in sheep

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ABSTRACT

This study was conducted to evaluate the therapeutic efficacy of new herbal supplements in sheep after induction of lactic acidosis. To achieve this goal, five sheep were used in two experiments in a crossover design with an interval of three weeks. In addition, 10 apparently healthy sheep were used as control. In the first experiment, lactic acidosis was induced with sucrose (18 gm / kg b.wt) and treated with Rumitone powder. In the second experiment, sheep were treated with both Rumitone and Meboliv AFS. Within 24 hrs of induction, the affected sheep showed anorexia, weakness, depression, lowered head, semisolid feces, distended abdomen, dyspnea, incoordination and recumbency. The acidotic sheep showed significant (p < 0.05) increase in respiratory and pulse rates; and significant decrease in temperature and ruminal movement. There was a significant increase (p < 0.05) in Hb conc., PCV%, RBCs, WBCs, granulocyte% and lymphocyte%. Biochemically, there were significant decreases in serum Na, Cl, Ca, albumin and total protein; whereas significant increases in K and P, AST and ALT, urea and creatinine were observed. The clinical, hematological, biochemical and ruminal parameters were significantly changed toward the control values post-treatment with Rumitone alone and combination of Rumitone and Meboliv AFS. Therefore, these herbal compounds are recommended for treatment of ruminal acidosis in sheep.

KEYWORDS: lactic acidosis, Meboliv, ruminal analysis, Rumitone, sheep.

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1. INTRODUCTION

Ruminal acidosis is defined as a decrease in the ruminal pH. The reduction in pH below the normal (optimum 6.8) has a significant impact on microbial activity, rumen function, and animal (Nagaraja productivity and health and Lechtenberg, 2007). Acute ruminal acidosis is one of the most dramatic forms of ruminal microbial fermentative disorders and in some cases is lethal in less than 24 hours (Gentile et al., 2004). Lactic acidosis is caused by sudden ingestion of grain or other carbohydrate-rich foods, highly fermentable in large quantities. The disease is characterized by appetite loss, depression and death. It is also known for ruminal overload, acute indigestion, acute compression of the rumen and indigestion for carbohydrates (Walker, 2006). Clinical signs vary with the severity of disease. Appetite and rumen movements are reduced or absent; diarrhea, dehydration, abdominal distension, tachycardia and tachypnea may occur, and laminitis can also be

biochemical changes (Jorg and Enemark, 2008; Patra et al., 1996; Schwegler et al., 2014). Prompt treatment of indigestion is necessary for improving However, treatment productivity. gastrointestinal diseases of ruminants poses significant challenge as the specific causes are unknown and effective therapies unavailable (Berschneider 2002). Further, the use of conventional treatment strategies is expensive and difficult for the treatment of clinical cases of indigestion in large animals. Hence, traditional remedies are being preferred world-wide (Maphosa and Masika, 2010). Among the traditional and complementary treatments, herbal drugs form the most common and important treatment modality (Ke et al., 2012). Herbal drugs and poly-herbal

observed. The animals remain recumbent and may

die due to severe circulatory failure (Neto et al.,

2005). Lactic acidosis was associated with

changes (Noura,

formulations are being preferred as they are cheap and safe (Arora et al., 1983; Nooruddin, 1983; Singh et al., 1989). Several studies have also proven the safety of herbal drugs intended to be used for treating various disease conditions (Devi et al., 2012) . Two recently introduced herbal preparations were evaluated for clinical efficacy in sheep suffering from ruminal acidosis, namely Rumitone and Meboliv AFS. Rumitone is herbal feed supplement helps maintain proper secretion of saliva and gastric juices and optimum rumenoreticular and intestinal movements. Meboliv also optimizes pancreatic and duodenal enzyme production and thus enhances the other processes of digestion as well.

This study aimed to monitor the clinical, hematological, biochemical changes, and ruminal juice examination associated with induced lactic acidosis in sheep. A further objective was to evaluate the effectiveness of Rumitone and Meboliv in the treatment of such a problem.

2- MATERIAL AND METHODS

2.1. Animals and study design:

Five healthy male sheep aged from 1-2 years old and weighting 50- 55 kg were used in this study in a crossover design with an interval of three weeks. Another 10 apparently healthy sheep were used as control. They were kept in clean disinfected pens, fed on green roughages and concentrates. All sheep were dewormed with a suitable anthelmintic. They were left for 2 weeks for acclimatization before the beginning of the experiment. During that period, sheep were subjected to a clinical investigation to be ensured healthy and free from any clinical abnormality.

2-2-Induction of ruminal lactic acidosis

An average dose of 18 gm/ kg b.wt sucrose was estimated to produce the classical clinical picture of the lactic acidosis according to (Afshin et al., 2011; Zein-Eldin et al., 2014). All sheep received sucrose after being fasted for 12h. The sucrose was mixed with 200 ml warm tap water, to make a suitable suspension, and was given using stomach tube in a single dose. The sheep under experiment were monitored for clinical changes up to the 24 hrs post induction after which blood and ruminal samples were collected for analysis.

2-3- Treatment protocols

In the first experiment, sheep with induced lactic acidosis was treated with Rumitone powder alone at a dose 1.5 gm per 50 kg Bwt.). In the second experiment (after 3 weeks of the first one),

sheep with induced lactic acidosis was treated with a combination of Rumitone powder at a dose 1.5 gm per 50 kg Bwt. and Meboliv AFS powder at a dose 5 gm per 50 kg Bwt. Rumitone was given at 8 A.M and Meboliv was given at 8 P.M. Both supplements were provided by the Indian Herbs Specialties Pvt. Limited (Dara Shivpuri, Nawads Road, Saharanpur, 247001 (U.P.), India.). The treatments commenced 24h after induction and continued for a week. Ringer lactate was given IV as a co-therapy to avoid mortalities.

2-4- Sampling

Two sets of blood samples were obtained from each sheep. The first set of samples was collected on labeled test tube containing 5mg K2 EDTA in concentration of 1 mg/1ml blood (Coles, 1986) as anticoagulant for determination of hematological parameters. The second set of blood samples was taken by allowing about 5 ml of blood to flow freely and gently over the inner surface of a clean and dry centrifuge tube. The samples were allowed to clot in slanting position at room temperature for about 2 hours then the samples were centrifuged at 3000 rpm for 10 minutes. The clear sera were aspirated carefully by automatic pipette and transferred into clear dry labeled Eppendorf tubes and stored at - 20°C till examination. Only clear non hemolyzed sera were used for the biochemical examination.

2-5- Clinical examination

Body temperature, respiratory rates, pulse rates, mucous membrane, and ruminal movement of the sheep were examined and recorded following the procedures described by Radostits et al. (2007). The degree of dehydration and the severity of diarrhea had been estimated by capillary refill time (Trefz et al., 2012).

2-6- Hematological examination

The erythrocytes count, hemoglobin conc., PCV%, total and differential leukocytes counts were determined by automatic hematology analyzer according to the method described by Jain (1993).

2-7- Biochemical examination

Total protein was determined in g/dL by colorimetric method according to the method described by Gornall (1949). Albumin was determined by colorimetric method according to the method that described by Young et al. (1975). Enzymatic colorimetric test used for the determination of urea in serum according to Eisenwiener (1976). Creatinine was determined by colorimetric test according to Tanganelli et al. (1982). Kinetic determination of ALT and AST were measured according to Klin (1972). Serum calcium, phosphorus, sodium, chloride, and potassium levels were determined spectrophotometrically by colorimetric method according to Jansen and Helbing (1991).

2-8- Ruminal juice analysis:

The ruminal juice was collected from all animals by using a simple ordinary stomach tube connecting with a suction syringe 50 ml capacity. Each sample (100 ml) was taken from different level of the ruminal contents in a clean dry and sterile flask. These samples were sieved and strained through 2 folds of sterile gauzes and immediately used for estimation of ruminal pH and determine physical characters (color, odor, consistency, and sedimentation activity test), protozoal activity, motility, and numbers. Ruminal fluid was preserved for further investigation as methylene blue reduction test.

2-9-Statistical analysis:

The data were statistically analyzed using one way analysis of variance with Dunnet's as a posthoc test as previously described (Bailey, 2008). We used SPSS version 16 software to conduct this analysis. Values were represented as means \pm standard error (SE). All differences were considered significantly different when P < 0.05.

3. RESULTS

3-1-The clinical examination:

The clinical examination of control sheep revealed good healthy condition which represented as good appetite, shiny eyes and normal defecation. The body temperature, pulse rate, respiratory rate and ruminal movement were within normal range (Table 1). The clinical signs of sheep after induction of lactic acidosis started within few hours after administration of sucrose.

The affected sheep showed depression, decrease feed intake, weakness; lowered head and with semisolid feces with slightly distended abdomen. There was significant increase (p<0.05) in respiratory, pulse rates, decrease in temperature and ruminal movement and the abdomen was slightly distended. Within 12-24 hours after

administration of sucrose, the affected sheep appeared inactive, dull and depressed, ruminal movements completely absent with diarrhea, dyspnea, incoordination and recumbency. The clinical parameters were significantly changed toward the control values after treatment with Rumitone alone and combination of Rumitone and Meboliv AFS (Table1).

3-2-Hematological examination:

There was significant increase (p < 0.05) in Hb concentration, PCV%, RBCs, WBCs. %, granulocyte% and Lymphocyte while monocyte% showed no significant change after induction of lactic acidosis compared to control and compared to values before induction. The Hematological parameters were significantly returned to the control values after treatment with Rumitone alone and combination of Rumitone and Meboliv AFS (Table 2).

3-3-Serum biochemical analysis:

There was significant increase in AST, ALT, urea and creatinine after induction of lactic acidosis in sheep. While albumin and total protein showed significant decrease There was a significant decrease in serum levels of sodium, chloride, and calcium while there was a significant increase in serum level of potassium and phosphorous.

The biochemical parameters were significantly changed toward the control values after treatment with Rumitone alone and combination of Rumitone and Meboliv AFS (Table 3, 4).

3-4-Ruminal juice examination:

The color, odor and consistency of ruminal juice were changed after induction of lactic acidosis in sheep. Sedimentation activity time (SAT) showed a significant increase. Microscopic examination of ruminal juice revealed presence of few numbers of live protozoa and their number showed significant decrease.

There was significant decrease in ruminal pH, and a significant increase in methylene blue reduction test (MBRT) after induction of lactic acidosis in sheep. These parameters were significantly returned back to the control values after treatment with Rumitone alone and combination of Rumitone and Meboliv AFS (Table 5).

 Table (1): Clinical parameters in control sheep, sheep with induced lactic acidosis and treated by Rumitone alone and in combination with Meboliv AFS.

			Rumitone	Rumitone & Meboliv AFS			
Parameter	Control	Before	After	After	Before	After	After
1 drameter	(n=10)	induction	induction	treatment	induction	induction	treatment
		(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
Temperatur e	39.12±0.03 a	$39.04{\pm}0.02^{b}$	38.64±0.02 a	39.02±0.02 ь	39.04±0.02 b	38.62±0.02 a	39.04±0.02 b
Respiratory rate/ minute	23.50±0.17 a	23.60±0.24 ^b	32.20±0.58 a	23.60±0.24	23.40±0.24 b	32.40±0.40	23.40±0.24 b
Pulse rate/ minute	79.60±0.22 a	79.40±0.245 b	89.20±0.49 a	79.40±0.24 ^b	79.60±0.24 b	89.60±0.40 a	79.60±0.24 ^b
Ruminal movement/ 2 minutes	2.40±0.16ª	2.40±0.24 ^b	0.00±0.00ª	2.20±0.20 ^b	2.60±0.24 ^b	0.20±0.20ª	2.60±0.24 ^b

Values with different superscript letters within the same row differed significantly at p < 0.05

Table (2): Hematological examination in control sheep, sheep with induced lactic acidosis and treated by Rumitone alone and in combination with Meboliv AFS.

	Rumitone Rumitone & Me							
Parameter	Control	Before	After	After	Before	After	After	
	(n=10)	induction	induction	treatment	induction	induction	treatment	
		(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	
Hb(gm/dl)	10.71±0.10 a	10.64±0.02ª	12.36±0.22 ^b	10.74±0.06ª	$10.62{\pm}0.04^{a}$	12.46±0.17 b	10.60±0.05ª	
PCV%	30.70±0.75 a	30.48±0.20ª	34.58±0.39 ^b	30.56±0.26 ^a	30.46±0.25ª	34.86±0.18 b	30.50±0.21ª	
RBCs(10 ¹² /l)	11.04±0.08 a	11.14 ± 8.09^{a}	12.86±0.13 ^b	11.22±0.14 ^a	11.18±0.08 ^a	12.80±0.11 b	11.22±0.11ª	
WBCs(10 ⁹ /l)	11.90±0.19 a	11.88±0.05ª	13.88±0.18 ^b	12.00±0.22ª	11.98±0.09ª	14.14±0.26 b	12.20±0.28ª	
Granulocyte %	17.09±0.03 a	17.10±0.04ª b	17.29±0.04 ^b	17.10±0.05ª b	17.04±0.04ª	17.40±0.08 c	17.18±0.10 ^a b	
Lymphocyte %	56.05±0.30 a	56.24±0.06 ^b	57.06±0.14°	56.06±0.12 ^a b	56.00±0.00 ^a b	58.44±0.24	55.60±0.24ª	
Monocyte%	5.45±0.18 ª	5.36±0.02 ^a	5.46±0.10 ^a	$5.34{\pm}0.09^{a}$	$5.38{\pm}0.02^{a}$	5.46±0.06 ª	5.46±0.05 ª	
Values with diffe	erent superscri	nt letters within	the same row	differed signifi	cantly at $n < 0.0$	15		

Values with different superscript letters within the same row differed significantly at p < 0.05

Table (3): Biochemical analysis in control sheep, sheep with induced lactic acidosis and treated by Rumitone alone and in combination with Meboliv AFS.

			Rumitone	Rumitone & Meboliv AFS			
Parameter	Control	Before	After	After	Before	After	After
Parameter	(n=10)	induction	induction	treatment	induction	induction	treatment
		(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
AST(U/1)	$51.20{\pm}0.80^{a}$	50.60 ± 0.60^{a}	71.76±6.83 ^b	51.32±1.15ª	50.40±0.68ª	75.61±8.46 ^b	50.82±1.01ª
ALT(U /1)	$18.80{\pm}0.16^{a}$	19.00 ± 0.45^{a}	34.47±2.69°	$19.81{\pm}1.29^{a}$	18.60±9.24 ^a	29.65±1.95 ^b	18.48 ± 1.13^{a}
ALB (g/dl)	3.30±0.058°	$3.36{\pm}0.02^{b}$	2.34±0.27 ^a	$3.33{\pm}0.05^{b}$	$3.26{\pm}0.07^{b}$	$2.51{\pm}0.17^{a}$	$3.28{\pm}0.16^{b}$
$\widetilde{TP}(g/dl)$	$6.57{\pm}0.10^{b}$	$6.72{\pm}0.02^{b}$	$5.13{\pm}0.20^{a}$	6.70 ± 0.03^{b}	$6.60{\pm}0.06^{b}$	$5.39{\pm}0.17^{a}$	$6.48{\pm}0.14^{b}$
Creatinine (mg/dl)	$0.93{\pm}0.01^{ab}$	0.84±0.02ª	1.40±0.05°	0.86±0.03ª	$0.90{\pm}0.00^{a}$	$1.62{\pm}0.07^{d}$	$1.03 {\pm}.04^{\text{b}}$
Urea (mg /dl)	23.40±0.45ª	23.00±0.32ª	30.62±0.99 ^b	22.63±1.0ª	23.00±0.32 ^a	33.17±3.15 ^b	22.13±1.42 ^a

Values with different superscript letters within the same row differed significantly at p < 0.05

			Rumitone	Rumitone & Mebolive AFS			
Paramete r	Control (n=10)	Before induction (n=5)	After induction (n=5)	After treatment (n=5)	Before induction (n=5)	After induction (n=5)	After treatment (n=5)
Ca (mg /dl)	9.63±0.12°	9.94±0.17°	8.46 ± 0.39^{b}	9.83±0.20°	9.56±0.13°	7.32±0.53ª	$9.19{\pm}0.36^{bc}$
Na (mEq /l)	149.10±0.2 8 ^b	149.60±0.4 0 ^c	140.80±1.1 6ª	149.00±0.71	149.00±0.00 bc	140.00 ± 0.8 4^{a}	146.20±1.8 3 ^b
K (mEq /l)	4.67±0.12 ^b	4.16±0.07 ^{ab}	4.86±0.08 ^{cd}	3.88±0.10 ^a	4.44±0.13 ^{bc}	$5.07{\pm}0.27^{d}$	4.16±0.17 ^{ab}
P (mg /dl)	5.03±0.07ª	5.50±0.22ª	7.42±0.77 ^{ab}	6.16±0.61ª	5.80±0.20ª	8.46±0.95 ^b	7.22±0.77 ^{ab}
Cl (mEq /l)	99.70±0.45 ^b	99.60±0.51 ^b	88.80±1.07ª	98.20±0.92 ^b	108.60±1.57 c	96.00±4.30 ^b	108.40±1.6 9°

Table (4): Biochemical analysis in control sheep, sheep with induced lactic acidosis and treated by Rumitone alone and in combination of Rumitone and Meboliv AFS.

Values with different superscript letters within the same row differed significantly at p < 0.05

Table (5): Physical properties and biochemical analysis of ruminal juice in in control sheep, sheep with induced lactic acidosis and treated by Rumitone alone and in combination with Meboliv AFS.

	Rumitone			Rumitone & Meboliv AFS				
Parameter	Control	Before	After	After	Before	After	After	
		induction	induction	treatment	induction	induction	treatment	
Color	-Olive green - Yellowish Brown	-Olive green -Yellowish Brown	-yellowish - Milky grey	-Olive green -Yellowish Brown	-Olive green -Yellowish Brown	-yellowish - Milky grey	-Olive green -Yellowish Brown	
Consistency	Slightly Viscous	Slightly Viscous	Watery	Slightly Viscous	Slightly Viscous	Watery	Slightly Viscous	
Odor	Aromatic	Aromatic	Soured	Aromatic	Aromatic	Soured	Aromatic	
S.A.T	$6.60{\pm}0.16^{a}$	$6.62{\pm}0.02^{a}$	41.20 ± 0.97^{b}	$6.40{\pm}0.29^{a}$	$6.62{\pm}0.02^{a}$	41.60±0.51 ^b	6.30±0.12ª	
pН	6.72±0.03°	6.78±0.02°	$5.82{\pm}0.07^{b}$	6.70±0.04°	6.76±0.02°	5.36±0.09ª	6.64±0.06°	
Methylene blue reduction test/ min	2.98±0.19ª	3.00±0.00ª	7.40±0.60 ^b	3.00±0.00ª	3.00±0.00ª	9.00±0.32°	3.40±0.24ª	

Values with different superscript letters within the same row differed significantly at p<0.05

Table (6): Microscopical examination of ruminal juice in control sheep, sheep with induced lactic acidosis and treated by Rumitone alone and in combination with Meboliv AFS. (Qualitative and quantitative method).

			Rumitone	Rumitone & Mebolive AFS			
Parameter	Control	Before induction	After induction	After	Before induction	After induction	After
		Induction	Induction	treatment	mauction	mauction	treatment
Protozoal Activity	+++	+++	+ or 0	+++	+++	+ or 0	+++
Protozoal countx10 ⁵ /ml	4.36±0.10°	$4.18{\pm}0.07^{\rm bc}$	$0.20{\pm}0.17^{a}$	$3.85{\pm}0.23^{b}$	4.36±0.11°	$0.00{\pm}0.00^{a}$	4.34±0.17°

+++ = highly motile and overcrowded. ++ =motile and crowded.

+ = sluggish motile and low number. 0 = no live protozoa.

Values with different superscript letters within the same row differed significantly at p < 0.05

4- DISCUSSION

Treatment of ruminal disorders represented a major concern in veterinary practice. The lactic acidosis, being the most common in small ruminant, recently attain great interest by several authors because it may lead to mortalities and economic losses. Therefore, it was crucial to integrate new therapeutic methodologies to control this problem. Our study tries to evaluate the curative efficiency of different herbal products recently introduced to the market.

Our results demonstrated that sheep with induced lactic acidosis showed signs of weakness, decrease of feed intake, depression, and stand with their head held lowered. The pulse rate and rates increased while ruminal respiratory movement decreased. These results coincided with Zein-Eldin et al. (2014). The low pH explains the increased respiratory rate with labored respiration by stimulation of respiratory centers (Radostits et al., 2007). Sheep suffering from acute rumen acidosis showed a ruminal stasis, diarrhea, dullness, incoordination and recumbency. The amplitude and frequency of ruminal contractions were progressively diminished with eventual stasis when the rumen pH reached 5.0 or less. The mechanism of rumen stasis could be attributed to the involvement of hydrogen ion receptors elsewhere in the gastro- intestinal tract and or the central inhibition of gastric center by the absorbed toxic amines and amides (Mohamed 2014). The observed diarrhea in most cases could be attributed to the fact that lactic acid is converted to sodium lactate which passed down to the intestinal tract producing an osmotic gradient and draws water into small intestine contributing to diarrhea, Hemoconcentration and dehydration (Blood and Radostits, 1989). All these disturbances could be attributed to changes in the pH of the rumen under the effect of excessive lactic acid production, histamine, methanol and its effect on the nerve centers (Radostits et al., 2007). Clinical examination of the sheep after treatment with Rumitone alone and combination of Rumitone and Meboliv AFS revealed significant alterations in the clinical parameters and marked improvement in the clinical signs, particularly the appetite, the ruminal movement, the pulse and respiratory rate and sheep regain normal demeanor.

Hematological examination revealed significant increase in Hb content, PCV %, WBCs, RBCs, granulocyte %, lymphocyte at 24 hours after induction of lactic acidosis. Our results agreed with that obtained by Noura (2012). The significant increase in erythrocytes, leukocytes and PCV

confirmed values that а degree of hemoconcentration and dehvdration occurred. which increased with the amount of fluids withdrawn from the extracellular fluid space into the rumen. The gastro- intestinal disorders during ruminal acidosis was aggravated, influencing the leukocytes count toward an increase (Radostits et al., 2007). Hematological picture was returned to the control values after the treatment with Rumitone alone and combination of Rumitone and Meboliv AFS.

Biochemically, there was a significant increase in AST and ALT. These results agreed with the results obtained by Abd El-Samee and Abdou (1997). The significant increase in AST enzyme 24 hours after induction of acidosis could be attributed to dehydration. On the other hand, the significant decreased values of total protein and albumin in diseased sheep agreed with the results obtained by Saber (1991). The significant decrease in these parameters may be due to the excretion and loss of these parameters in the intestinal lumen with diarrhea or due to anorexia (Cao et al., 1987). The significant increase in serum urea and creatinine were due to dehydration and oligurea which occurs to compensate the fluid losses of the body (Owens et al., 1998).

With regard to serum minerals and electrolyte changes, there were significant decrease in serum levels of calcium, sodium and chloride while potassium levels and phosphorus were significantly increased. These results agreed with that obtained by Jorg and Enemark (2008). The decreased levels of serum sodium and chloride may be due to the shift of these electrolytes by osmolarity from the blood to the hypertonic rumen (as high lactic acid increase hypertonicity in rumen) or due to their losses (Cl and Na) in lactic acidosis associated with diarrhea (Jorg and Enemark, 2008). The observed hyperkalemia could be related to hemoconcentration, dehydration and acidemia. The decrease in calcium might be attributed to a temporary malabsorption due damaged mucosa of intestine secondary to acidosis (Radostits et al., 2007).

Regarding the ruminal fluid analysis, the color varied from yellowish to milky grey color, sour odor, watery in consistency and that SAT time was significantly increased. These results were consistent with those reported by Khaled and Baraka (2011) and Mohamed (2014). The milky – grey color of ruminal juice in diseased cases could be arisen from excessive sucrose with abnormal fermentation. The watery consistency and sour odor could be attributed to excessive production of lactic acid (Rodostits et al., 2000). SAT was used as guide to evaluate the activity of microflora and the prolongation of that time suggest inactivation of ruminal microflora secondary to rumen acidosis (Kimberling, 1988). Physical properties of ruminal juice after treatment was improved in which color became olive green or yellowish brown, the odor become aromatic, the consistency become slightly viscous and the SAT was shorter than before treatment. Microscopical examination of ruminal juice after induction of lactic acidosis showed marked reduction in the activity and count of ruminal protozoa. These results agreed with Zein-Eldin et al. (2014) and Mohamed (2014). Death of microflora may be due to decrease of ruminal pH and increase level of lactic acid as the microflora accustoms the life in neutral media 6.2-7.2 (Steen, 2001). These results returned to normal after treatment by Rumitone alone and combination from Rumitone and Meboliv AFS. The Methylene blue reduction test (MBRT) was used to evaluate the activity of microflora (Mohamed 2014). As the rumen pH decreases, the number of lactate producing bacterial species such as Streptococcus bovis may increase than the number of the lactate - utilizing species, leading to accumulation of lactate in the rumen. This indicates the inverse relationship between lactic acid concentration and pH as recorded by Henning and Hagg (2010). The prolonged MBRT indicate inactivity of the ruminal microflora as previously indicated (Mohamed 2014).

Probiotics may stimulate appetite and improve the nutrition by the production of vitamins, detoxification of compounds in the diet, and by the breakdown of indigestible compounds into simpler compounds (Hemaiswarya and Ravikumar, 2013). Rumitone containing natural enzymes, lactobacillus probiotics and digestive stimulants that optimizes the population and activity of ruminal and intestinal microflora as well as enzymatic activity for efficient cellulose breakdown and optimum digestion, absorption and utilization of carbohydrates, proteins and lipids leading to improved feed conversion and farm productivity. Rumitone also helps maintain right balance between beneficial bacteria and pathogens in the intestines for better gut health. This explain the changes that happened after treatment, there was improvement in feed intake, as the animal began to eat 24 hours after treatment, also there was increase in ruminal movement, as the pH returned back to the control level. There was increase in the protozoal count and the field was crowded with motile protozoa so the supplementation of optimum Rumitone facilitates digestion. absorption and utilization of feed leading to improvement in feed conversion efficiency and

farm productivity Rumitone is unique as it helps to limit the possibilities of off-feed conditions and energy deficit. Meboliv consists of some selected herbal plants such as andrographis paniculata, eclipta alba, phyllanthus niruri etc, in the desired proportions which optimizes liver function and thus the secretion and flow of bile and metabolic activities. Meboliv also optimizes pancreatic and duodenal enzyme production and thus enhances the other processes of digestion as well, the use of Meboliv not only ensures optimal liver function but also, because of its effect on bile secretion, optimizes digestion and absorption of carbohydrates, fats and fat soluble nutrients as well as minerals and vitamins. This might explain the changes that happen after the treatment with Rumitone and Meboliv.

5- CONCLUSION

In a conclusion, the induced lactic acidosis is sheep is associated with changes in the clinical, hematological, biochemical and ruminal fluid parameters mostly 24 hrs post-induction. However, the use of herbal feed supplementation with Rumitone alone or combined with Meboliv significantly restored these changes toward the control values. Therefore, these compounds could be included in the prescription of therapy for rumen acidosis in sheep. Further studies are required for validation of the use of both compound alone and in combination in different animal species for treatment of digestive disturbances.

5- REFERENCES

- Abd El-Samee, A.A., Abdou, 1997. Investigation on the influence of rumen acidosis on blood chemistry and some rumen liquor parameters in goats. Egypt. vet. Med. Assu. 57, 509-522.
- Afshin, J.D., Mohammed, R.H., Zahra, K.D., 2011. ECG changes in acute Experimental Ruminal Lactic Acidosis in sheep. Veterinary research forum 2, 203- 208.
- Arora, S.P., Thakur, S.S., Tripathi, A.N., Chhabra, A., 1983. Influence of Galog on digestibility and milk production of Karan Swiss cows. Indian Veterinary Journal 9, 46-50.
- Bailey, R.A., 2008. Design of Comparative Experiments. Cambridge University, 116-128.
- Berschneider , H.M., 2002. Complementary and alternative veterinary medicine and

gastrointestinal disease. Clin Tech Small Anim Pract 17, 19-24.

- Blood, D.C., Radostits, O.M., 1989. Veterinary Medicine. 7th ed., Bailliere, Tindall, London, U.K.
- Cao, G.R., English, P.B., Filippich, L.J., Inglis, 1987. Experimentally induced lactic acidosis in the goat. Aust.Vet. J. 64, 367-370.
- Coles, E.H., 1986. Veterinary Clinical Pathology. 4th ed., W.B. Saunders Company, Philadelphia, U.S.A.
- Devi, S.P.R., Adilaxmamma, K., Srinivasa Rao, G., Srilatha, C.H., Alpha Raj, M., 2012. Safety Evaluation of alcohoic extract of Boswelliaovalifoliolata stembark In rats. Toxicology International 19, 115-120.
- Eisenwiener, H.G., 1976. J. Clin. Chem. Clin. Biochem 14, 261-264.
- Gentile, A., Sconza, S., Lorenz, I., 2004. D-lactic acidosis in calves as a consequence of experimentally induced ruminal acidosis. Journal of Veterinary Medicine 51, 64-70.
- Gornall, A.J., 1949. Biol Chem. 177 751.
- Hemaiswarya, S., Ravikumar, R., 2013. Mechanism of Action of Probiotics. Braz. Arch. Biol. Technol. 56, 113-119.
- Henning, P.H., Hagg, F.M., 2010. The potential of Megasphaera elsdenii isolates to control ruminal acidosis. Animal feed Sience and Technology 157, 13-19.
- Jain, N.C., 1993. Essential of veterinary Hematology. 5th Ed. Lea and Febiger, Philadelphia.
- Jansen, J.W., Helbing, A.R., 1991. Eur.J. Clin. Chem. 29 197-201.
- Jorg, M.D., Enemark, 2008. The monitoring, prevention and treatment of sub acute ruminal acidosis (SARA): A review. TheVeterinary Journal 176, 32-43.
- Ke, F., Yadav, P., Ju, L.Z., 2012. Herbal medicine in the treatment of ulcerative colitis. Saudi J Gastroenterol 18, 3–10.
- Khaled, N.F., Baraka, T.A., 2011. Influence of TOMOKO® (Direct fed Microbials) on productive performance, selected Rumen and Blood Constituents in Barky Finishing Lambs. Journal of American Science 7, 9.
- Kimberling, C.V., 1988. Jensen and Swiff's disease of sheep. 3rd ed., Lea and febiger. Philadelphia, USA.
- Klin, Z., 1972. Chem.u.Biochem.8 (1970) 658 and 10(1972), 182.
- Maphosa, V., Masika, P., 2010. Ethnoveterinary uses of medicinal plants: a survey of plants used in the ethnoveterinary control of gastrointestinal parasites of goats in the

Eastern Cape Province, South Africa. Pharm Biol 48, 697–702.

- Mohamed , A.E.A., 2014. Studies on ruminal disorders in sheep. Assiut Vet. Med.J. 60, 12-17.
- Nagaraja, T.G., Lechtenberg, K.F., 2007. Acidosis in feedlot cattle," Veterinary Clinics of North America. Food Animal Practice 23, 333–350.
- Neto, M.E.G., Afonso, J.A.B., Mendonca, C.L., Almeida, M.Z., 2005. Estudo clínico e características do suco ruminal em caprinos com acidose láctica induzida experimentalmente. Pesquisa Veterinária Brasileira 25, 73-78.
- Nooruddin, M., 1983. Clinical trial of Himalayan Batisa in loss of appetite of cattle. Indian J Anim Sci 8, 69-70.
- Noura, E.A.A., 2012. Studies on some digestive system disturbances in sheep and goats with trials for treatment. PhD. Thesis, Fac. of Vet. Med., Zagazig University.
- Owens, F.N., Secrist, D.S., Hill, W.J., Gill, D.R., 1998. Acidosis in cattle: A review. Journal of the American Society of Animal Science 76, 275-286.
- Patra, R.C., Lal, S.B., Swarup, D., 1996. Biochemical profile of rumen liquor, blood and urine in experimental acidosis in sheep. Small ruminant Research 19, 177-180.
- Radostits, O.M., Gay, C.C., Hincheliff, K.W., Constable, P.D., 2007. Veterinary Medicine A textbook of the diseases of cattle, horses, sheep, pigs and goats. Tenth Edition. B. Saunders, London, New York, Philadelphia, Sydney and Toronto.
- Rodostits, O.M., Gay, C.C., Blood, D.C., Hinchkliff, K.W., 2000. Rumen Acidosis. In: pp. 293-303. Veterinary Medicine, 9th Edition: Saunders, Elsevier, London.
- Saber, 1991. Some studies on indigestion in sheep. M.V.Sc. Thesis to Fac. of Vet. Med., Cairo, University.
- Schwegler, E., Silveira, P.A.S., Montagner, P., Da Silva, V.M., Rabassa, V.R., Schneider, A., Roos, T.B., Pfeifer, L.F.M., Schmitt, E., Del Pino, F.A.B., Correa, M.N., Gil-Turnes, 2014. The use of sodic monensin and probiotics for controlling subacute ruminal acidosis in sheep Braz. J. Vet. Res. Anim. Sci., São Paulo 51, 324-332.
- Singh, N., Rajesh, K., Akbar, M.A., 1989. Biochemical and microbial changes in rumen of anorexic buffaloes. J Vet Physiol Allied Sci 8, 36-44.
- Steen, A., 2001. Field study of dairy cows with reduced appetite in early lactation : clinical

examination , blood and rumen fluid analysis. Acta. Vet. Scand. 42, 219-228.

- Tanganelli, E., Principe, L., Bassi, D., Cambiaghi, S., Murador, E., 1982. Clin. Chem. 28, 1461-1464.
- Trefz, F.M., Lorch, A., Feist, M., Sauture, C., Louis, Lorenz, I., 2012. Metabolic acidosis in neonatal calf diarrhea- clinical finding and theoretical assessment of a simple treatment protocol. J Vet Tentern. Med 26, 162-170.
- Walker, B., 2006. Grain poisoning of cattle and sheep. Prime Facts 330, 1-4.
- Young, D.S., Pestaner, L.C., Gibberman, V., 1975 Effects of drugs on clinical laboratory tests. Clin Chem 21, 1D-432D.
- Zein-Eldin, M.M., Ghanem, M.M., Abd El Raof, Y.M., El Attar, H.M., 2014 Clinical, Haematobiochemical and ruminal changes during the onset and recovery of induced lactic acidosis in sheep. Biotechnology in Animal Husbandry 30, 647-659.